AUXOGRAPHIC MEASUREMENT OF SWELLING OF BIOCOLLOIDS AND OF PLANTS

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(WITH TWO FIGURES)

The chief purpose of this article is to describe the methods which have been used in the study of colloidal preparations, the reactions of which might furnish a physical basis for the interpretation of growth in plants, and to recapitulate some of the features of swelling of these substances as yet undescribed or but little known.

The investigation of growth in plants involves a measurement of the unsatisfied hydration capacity of living cell masses, and also determinations of the total swelling capacity of desiccated material. In both cases the minute masses of colloids constituting the protoplasts are inclosed in thin walls with a low stretching coefficient. Furthermore, the living cells are in a condition of varying turgidity, dependent upon the osmotic activity of the vacuolar solutions, upon the permeability of the external layer of the protoplasm, and also upon the structure of the walls. Desiccated cell masses have lost the capacity of turgidity as ordinarily known, but may still show some osmotic activity by the differential action of the dead wall (as may other colloids), while the protoplasts have undergone changes due to the action of salts and acids in the concentration of cell saps which accompanies desiccation.

It is not possible to reproduce the mechanical structure of cell masses by artificially compounded biocolloids, so the experimenter must be content to ascertain the general composition of the protoplasm, bring the main constituents together in the form of a jelly, dry this to thin plates, and measure the action of sections of it in solutions of a kind and concentration which would give effects similar to those encountered in living matter.

Physiologists concerned with life in animals dealing with a protoplasm consisting chiefly of proteins and lipins with a characteristic metabolism, have found in gelatine and in the soaps material

which furnishes many valuable homologies. These are in imminent danger of being overworked, however. Not only has gelatine been taken to simulate protoplasm in general, but physicists have committed a similar error of taking the behavior of gelatine as universal for colloids.

Not much progress had been made in the attempt to determine the physical basis of growth in plants before it became plainly obvious that the relations of plant protoplasm to H ion concentration, acidity, temperature, and other conditions diverged widely or were directly the reverse of the action of gelatine.

In seeking other material in a colloidal state which might by its hydration, swelling coefficient, etc., simulate the plant, recourse was had to actual analyses of plants as made in connection with studies in desiccation, starvation, etiolation, and as part of the work on the carbohydrate metabolism of plants by SPOEHR.[1]

The possible importance of the pentoses as a factor in the mechanism of hydration and growth was evident at once. A knowledge of the manner of formation and occurrence of these sugars and of their condensation products, the pentosans (mucilages, slimes, gums, etc.), is necessary in order to understand the rôle they play in the cell.

The metabolism of the plant is predominantly carbohydrate, and the protoplasm may be taken to contain solutions of sugars at all times. Included among the numerous possible changes, it is known that in the depletion of the water content of a plasmatic mass by a general loss from the cell, or by lowered hydration capacity of the colloid by the action of any agent, polysaccharides which have but little imbibition or hydration capacity are reduced to pentosans or mucilages which have a relatively enormous capacity for taking up water. Dextrose, starch, wall material, etc., may be involved in these conversions, and, when masses of material are affected, layers or globules of mucilage may be formed which may react to microchemical tests. It is noted, however, that visible masses of mucilage are of less importance in the mechanism

[1] SPOEHR, H. A., Carbohydrate economy of the cacti. Publ. no. 287. Carnegie Inst. Wash., p. 44.

———, The pentose sugars in plant metabolism. Plant World **20**:365. 1918.

of the cell. The pentosans play their most important part when they are in the form of minute particles in the colloidal mesh of living matter, in which they are still subject to positive but slow changes in metabolism. According to MACDOUGAL, RICHARDS, and SPOEHR,[2] the conversion of other sugars into the pentosans, greatly increasing the hydration capacity of the protoplasm, is the basis of the origination of the xerophytic and succulent types of vegetation.

These facts warrant a procedure in the study of swelling and growth in which various pentosans and pentosan-protein mixtures were subjected to the action of solutions, applied in a concentration and with variations and alternations parallel to occurrences in the cell.

The method of measuring the water capacity of colloidal material includes the following features:

1. Suitable solutions or suspensions are poured at temperatures of 30 to 40° C. into shallow molds to form a jelly.

2. The plates thus cast are dried in a small chamber with a high relative humidity, to a thickness of 0.1 to 0.25 mm.

3. Trios of sections 3×5 mm. in area are placed in dishes of a capacity of 30 cc., a triangular piece of thin glass is placed over the sections, and the vertical swinging arm of an auxograph is seated in the center of the glass. Any change in thickness of the sections moves the levers and moves a pen on a recording sheet carried by the drum.

4. Solutions, the effect of which on the hydration capacity of the sections are to be tested, are poured into the dishes. In nearly all of the experiments in the special range of biological relations it is advisable to renew or replace the solutions at certain intervals.

5. Temperature relations are of the greatest importance, and the record is taken by mercurial thermometers, the bulbs of which are in dishes of liquid similar to those of the experiment.

[2] MACDOUGAL, D. T., and SPOEHR, H. A., The origination of xerophytism. Plant World **21**:245. 1919.

MACDOUGAL, D. T., RICHARDS, H. M., and SPOEHR, H. A., The basis of succulence in plants. BOT. GAZ. **67**:405. 1919.

6. Every measurement obtained by this method is an average of the action of three sections, and has the value of an average. Any value showing notable departures from expectancy should be repeated.

7. Measurements of the swelling of sections by the auxograph give variations in thickness, and serve as direct indices of total changes in volume in plates of agar and agar mixtures, as this material has a strict tendency to return to its original form. Sections of gelatine swell in all dimensions no matter how the plates are cast.

It was desirable to use the best known and most available pentosans or hemicelluloses, and agar-agar, acacia gum, tragacanth, mesquite gum, cherry gum, and mucilage of *Opuntia* were selected for the tests. According to information furnished by H. NAKANO of the Botanical Garden of Tokyo, agar is prepared chiefly from the algae *Gelidium amansii* Lamour, *G. pacificum* Okam, *G. linoides* Kütz., and *Pterocladia capillacea* Born. et Thur., while some material of *Gelidium subcostatum* Kütz., *Ceramium Boydenii* Gepp., *Campylaephora hypenaloides* Y. Ag., and *Acanthopeltis japonica* Okam, etc., may be included. The process includes washing in fresh water, decoloration in the sun, milling, boiling, filtering, maceration in sulphuric or acetic acid, freezing, and drying. Modernized methods simplify this treatment somewhat. Salts, amino-acids, etc., may be present in the final product, and, as it was desirable to reduce the amount of all such subtances, the interest of E. R. Squibb and Sons was obtained, and a purified product was made by the following procedure.[3]

A good grade of commercial agar was dissolved in distilled water and jacketed with superheated steam. The viscous solution produced thereby was filtered clear through a thick mass of steam-jacketed paper pulp, under diminished pressure. The clear solution of agar was dialyzed for about 10 days in a steam-jacketed bath containing running distilled water. After removal of diffusible carbohydrates and salts by the dialysis through parchment paper

[3] For further information concerning the origin and preparation of similar products see SWARTZ, M. D., Nutrition investigations on the lichens, algae, and related substances. Trans. Conn. Acad. Arts and Sci. **16**:247–382. 1911.

of the quality usually employed in the serum industry, the clear solution was slowly poured in a fine stream or spray into 10 times its volume of neutral acetone. The agar was precipitated in the form of fine shreds. It was subsequently extracted with hot absolute acetone, absolute alcohol, and absolute ether. The final product was ground to a granular powder in a porcelain ball-mill with porcelain balls. The resulting preparation contains only a trace of nitrogen and a trace of ash.[4]

Samples of this agar made up as a 2.5 per cent solution with distilled water had a light brown tinge, dissolving completely within an hour at about 100° C. It was found that the material made up as a 0.75 per cent solution, when poured into a test-tube, formed a "slant" which kept in place when the tube was set in an upright position at 15° C. for 2 weeks. Layers 1 cm. deep in small flasks retained their form when the flasks were inverted in some instances. Some water was separated, gathering on the upper part of the tube or flask, and the appearance of same on the surface of the agar suggested syneresis. Tests of this agar by the colorimetric process of DUGGAR gave it a hydrogen ion concentration denoted by $P_H = 6.5$.

The mucilage of *Opuntia*, cherry gum, mesquite gum, *Acacia*, and tragacanth gum was extracted or dissolved in water (nearly always showing a solid residue), precipitated with alcohol, filtered, and dried in a desiccator. These substances go into solution at lower temperatures than agar, and differ from it in many important features, as may be seen in the table of swelling reactions.

Special preparations were also made of salt-free water-soluble albumins from oats, wheat, the common bean, soy bean, and castor bean, as well as of lipins and amino compounds, by methods commonly used and need not be described here. The gelatine used was the "bacto-gelatine" now available in quantity.

The measurement of the swelling of these colloids must take into account the structural features resulting from dehydration. Sections or plates of agar, for example, tend to return to the form and dimensions of the layer of watery gel from which they were produced. This is not true of gelatine, as will be described later,

[4] The description of this process was furnished by Dr. ISAAC HARRIS.

nor can it be said of the other mucilages or gums already mentioned as they go into solution from the surfaces of the sections; but sections in which agar forms as much as half may be assumed to return to the original in nearly all work, with a change of not more than 2–5 per cent in area (fig. 1).

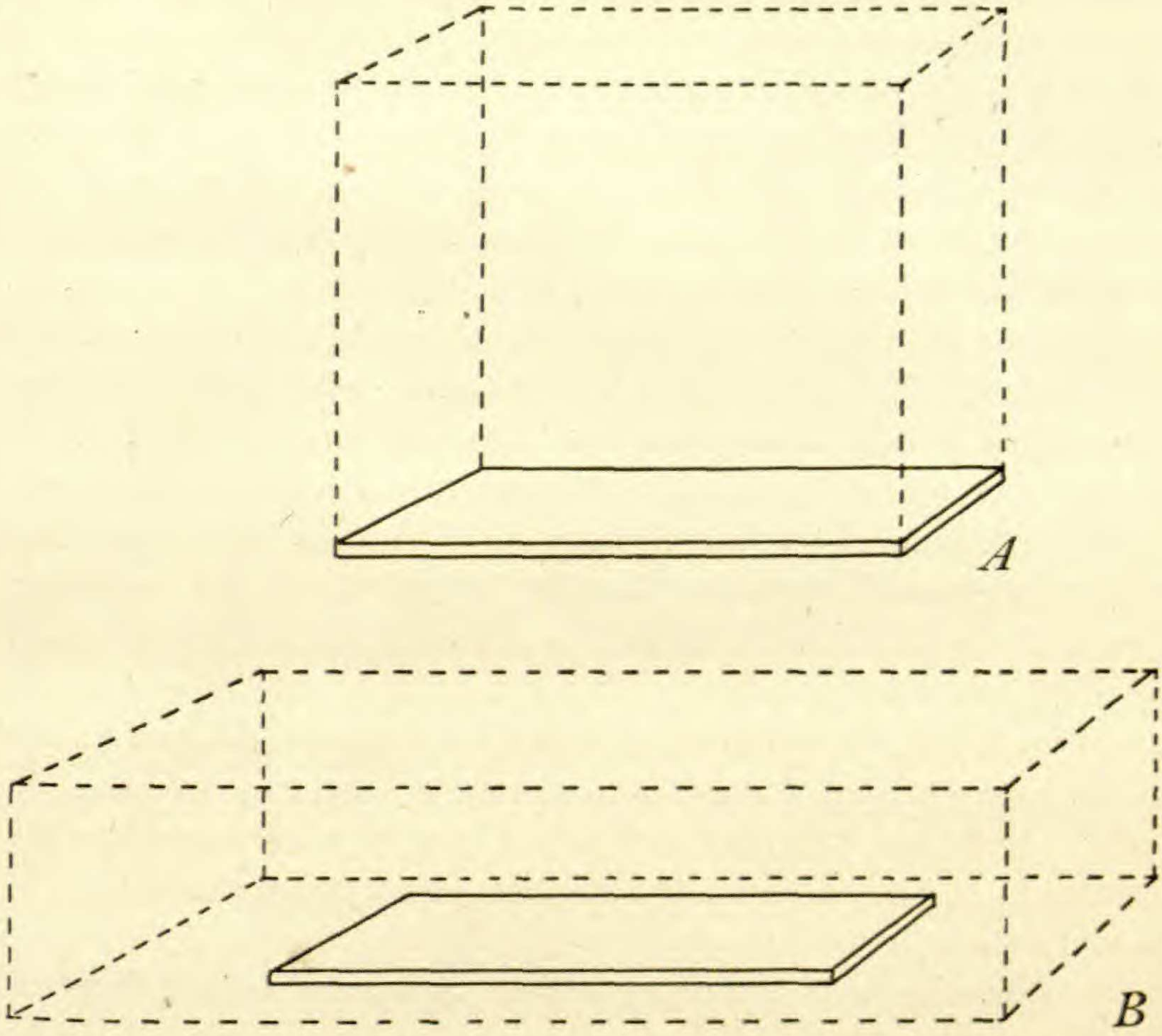

FIG. 1.—Diagrams illustrating action of dried plates of agar and gelatine in swelling: in *A*, section of agar, represented by thin plate forming bottom of figure, swells to larger block by increase almost wholly in thickness; in *B*, gelatine plate represented by small inner figure increases not only in thickness but also in length and breadth to form larger block of jelly.

In view of these facts, it is evidently desirable to cast plates and dry sections in such manner that the dried sheet of material will have the areal dimensions of the original, and that all of its shrinkage will have taken place in such manner as to reduce the thickness only. The preparation of such plates may be done simply by pouring warm solution on a glass plate, where it hardens,

dries, and adheres. If it does adhere in such manner as to prevent areal shrinkage, it generally is to be removed with difficulty and may be destroyed in the process.

An improved method of making such plates is briefly as follows. First a sheet of gold foil is laid out on a leveled glass plate on a table. Blocks of glass or of some non-corrosive metal are placed on the margins of the gold. The warm agar is poured into this cell in a 2.5 per cent solution, at 40° C., and in cooling to 18 or 20° C. it sets, and the blocks may be removed. The gel now stands on a base of gold foil, and unless anchored at the margins will shrink in all dimensions as it dries, a thing which specifically is to be prevented. To do this a little warm solution of agar is run around the agar plates which on cooling cements the margin to the glass. The preparation is now set in a dehydrating chamber with a humid atmosphere and subjected to the action of an electric fan for 40 hours. At the end of this time it has come down to a plate about one-fortieth of its original thickness, and may be detached from the plate by cutting away the marginal portion. Properly made, such plates are even as to thickness and swelling qualities.

When albumin, gelatine, or other albuminous substances are to be mixed with the agar, the solution of the latter is cooled to 40° C., and then the other substances in a liquefied condition are poured in and stirred for a few minutes before the plate is poured, which should be done at about 35° C.

The process puts the experimenter in possession of a sheet of dried material, preferably between 0.15 and 0.20 mm. in thickness, and after the margins are trimmed away with the scissors the sheet will probably have a surface of about 7×12 cm. As indicated, these sections may be about 3×5×0.16 mm., with a volume of 2.4 cu. mm., and the trio in a dish have a total of 7+cu. mm., into which a measured amount of solution is poured and replaced as the experiment demands. If thinner sections are used, their area should be reduced.

The auxograph consists essentially of a compound lever set with a vertical swinging arm which rests on the triangular glass plate covering the sections in the dish, and its use in measuring

the swellings is a means of recording phenomena not to be evaluated in any other manner[5] (fig. 2).

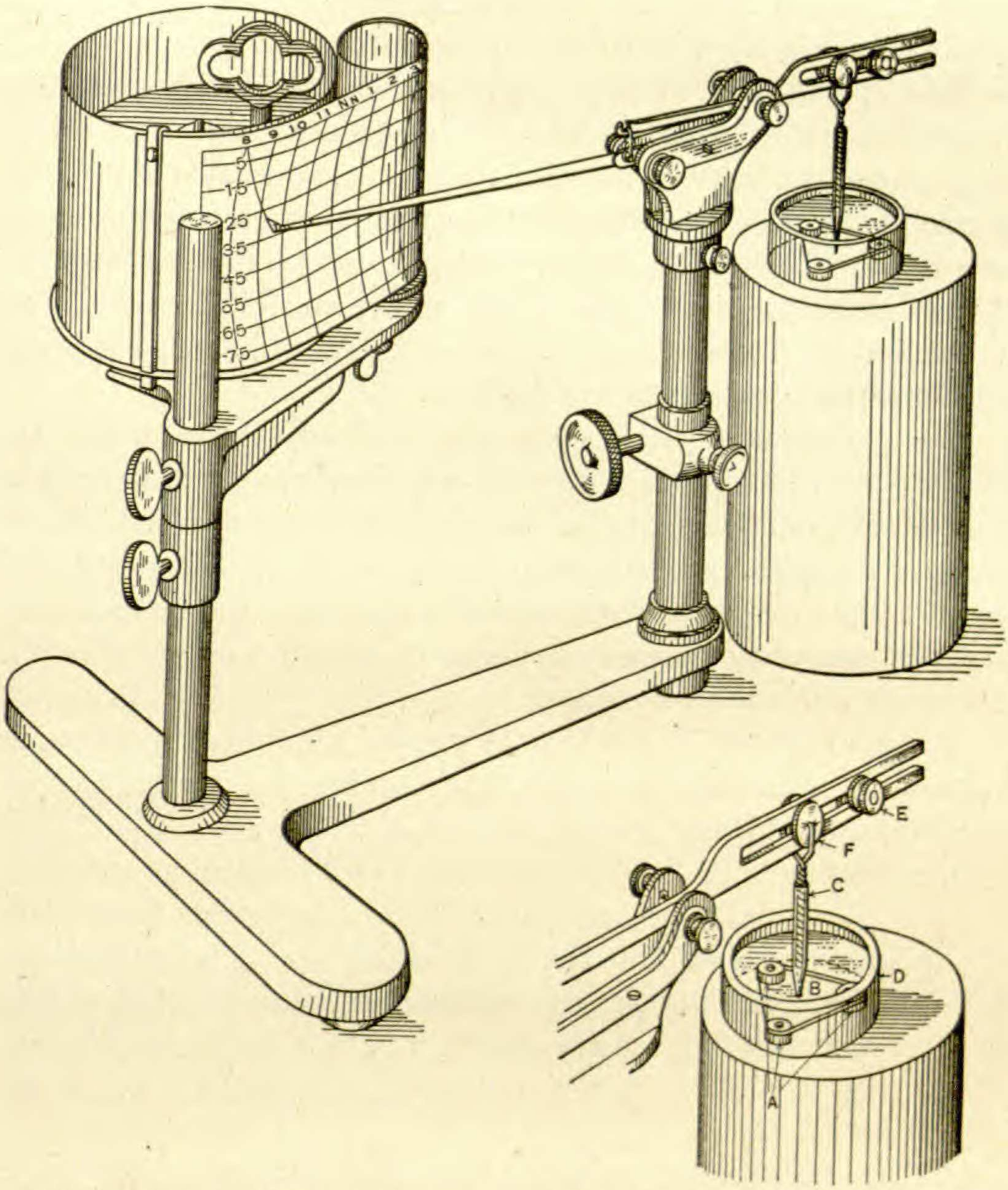

FIG. 2.—Auxograph arranged to record changes in thickness of trio of sections of colloidal material; tip of pointed glass tube, sealed to vertical swinging arm *C* (smaller figure), rests in socket in center of thin triangular glass plate *B*, which lies on sections *A;* expansion of sections pushes this arm upward (adjustment at *F* placed to give an amplification of 20 in cut), compound lever being arranged to give a downward movement of pen; record slip, daily or weekly, 8 cm. wide, is ruled to mm. (not shown in cut). Illustration made to show action of trio of sections of agar in water at end of 1.5 hours.

[5] For description of this apparatus see MACDOUGAL, D. T., Hydration and growth. Publ. no. 297. Carnegie Inst. Wash. 1919.

Thus, for example, instead of the common method of taking the total gross volume of a colloid in a dish or test-tube, the auxograph calibrates the action of sections like those of agar, which swell almost wholly in one axis (and a related heterotropic swelling is highly probable in all protoplastic action), and records the continuous rate of swelling. This gives the experimenter exact information on many features, of which the practical cessation of swelling is one of the most important. Thus some of the records show a swelling at a decreasing but notable rate for as long as 20 days. The termination of the experiment at the end of the second or even the tenth day would have eliminated some of the most striking and important features of the reaction.

Such continuous records are also necessary in following the action of solutions in which the mass relations are such as to require renewal of the liquids, and in the study of plates in which amino compounds, salts, etc., have been incorporated. Of other features, by no means the least advantageous is the use of the same instrument in measuring changes in volume by growth swelling of living and dried cell masses of plants.

A set of results of swelling of various biocolloids is given in table I, in which the increases, first obtained in percentages of the original thickness of the dried material, are converted into figures relative to the swelling in water which is taken as 100.

The P_H values were calculated from colorimetric tests after the indicator method perfected by DUGGAR,[6] soy albumin showing a value of 6.2 in a 0.5 per cent solution, *Opuntia* mucilage 5.8 in a 1 per cent solution, cherry gum 5.1 in a 1 per cent solution, acacia gum 5.1 in a 1 per cent solution, and gelatine 5.2 in an 8 per cent solution.

These biocolloids are to be regarded as intimately mixed particles, strands, webs, or globules of pentosans and of proteins, as these substances do not unite and are not mutually interdiffusible. The combinations of material were so arranged as to

[6] DUGGAR, B. M., and DODGE, C. W., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. 6:61–70. 1919.

DUGGAR, B. M., Refinements in the indicator method of hydrogen ion determination, Rept. Dept. Bot. Research, Carnegie Inst. Wash. 1919.

give illustration of the special character or dissimilarity of constitution of these mucilages when used to replace part of the agar. First it is to be seen that in the biocolloids in which acacia, cherry, and mesquite gum replace one another, the reactions to high H ion concentration in the acid and to potassium hydroxide are not widely different in total amount. The coefficient of increase in

TABLE I

RELATIVE SWELLING OF BIOCOLLOIDS IN ACIDS, HYDROXIDES, SALTS, AND WATER AT 15° IN 0.01 N CONCENTRATION; INCREASE IN WATER GIVEN IN PERCENTAGES OF ORIGINAL DRIED THICKNESS OR VOLUME

Material	Parts	KOH	NH_4OH	KNO_3	HNO_3	HCl	Water
	P_H	11.99	10.61	6.6	2.01	2.01	
Agar	10	33	83	39	44	44	(1800)
Gelatine	10	200	197	80	245	300	(1570)
Agar	8	50	45	45	32	23	(2000)
Soy bean albumin	2						
Agar	4	19	25	47	22	22	(2785)
Opuntia mucilage	4						
Soy bean albumin	2						
Agar	4	29	41	58	26	28	(2415)
Cherry gum	4						
Soy bean albumin	2						
Agar	4	33	36	67	31	29	(2100)
Acacia	4						
Soy bean albumin	2						
Agar	4	110	91	96	48	48	(1100)
Acacia	4						
Gelatine	2						
Agar	3	75	90	67	50	56	(1200)
Acacia	3						
Soy bean albumin	2						
Gelatine	2						

potassium nitrate does vary widely, however. The special effects in ammonium hydroxide are discussed elsewhere.[7]

When these mixtures are viewed as homologues of cell masses or of plant protoplasm, that containing the *Opuntia* mucilage

[7] MACDOUGAL, D. T., and SPOEHR, H. A., The effect of organic acids and their amino compounds on the hydration of agar and on a biocolloid. Proc. Soc. Exper. Biol. and Med. **16**:33. 1919.

assumes a special interest, as the total swelling in water is greatest of all of the combinations, and the depressing effects of acid, hydroxide, and salt are very marked. These features would be characteristic of a plant capable of a wide range of water content and sensitive to changes in the sap, as is known to be the case from studies of the growth of *Opuntia*.

The substitution of gelatine for albumin, or its addition to a mixture increases swelling in acid, salt, and hydroxide as would be expected, and lessens the total swelling in water. These and other available data may be profitably construed in many directions in the interpretation of growth phenomena.

The methods of preparation and measurement of swelling of colloids described have served to confirm and extend knowledge of the behavior of agar, albumin, gelatine, and of mucilages, and to fix upon pentosan-protein mixtures which swell in a manner similar to cell masses of plants. The use of the auxograph has made it possible to compare the nature, extent, and duration of these changes with variations in volume of growing cell masses.

The casting and desiccation of colloidal plates in such manner that shrinkage and swelling takes place unequally in different axes, and the measurement of such differential swelling also furnishes some evidence which may be of value in interpreting the changes in form, etc., of the special bodies of the protoplast which accompany and mark the morphological crises of the cell.

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